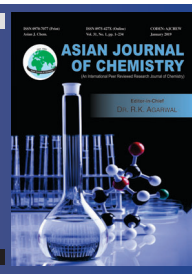
Asian Journal of Chemistry; Vol. 31, No. 12 (2019), 2729-2732

ASIAN JOURNAL OF CHEMISTRY

https://doi.org/10.14233/ajchem.2019.22161



A Novel Synthesis, Characterization and Biological Studies of Ferrocenyl Substituted Pyrazoles

MANOJ KUMAR[1,*], SHASHI SHARMA[1], HARDEEP SINGH TULI[2], RAJSHREE KHARE[3] and VINIT PARKASH[1]

[1]Department of Chemistry, Maharishi Markandeshwar University, Sadopur-Ambala-134003, India
[2]Department of Biotechnology, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala-133207, India
[3]Department of Chemistry, Maharishi Markandeshwar (Deemed to be University), Mullana-Ambala-133207, India

*Corresponding author: E-mail: manojraju27@gmail.com

Received: 28 April 2019; *Accepted*: 5 June 2019; *Published online*: 16 November 2019; AJC-19611

It has been discovered that ferrocenyl substituted heterocyclic compounds have wide scope of restorative methodology. The combination of ferrocenyl substituted pyrazole is the new class in these compounds with upgraded natural activity. This work center around blend of ferrocenyl substituted pyrazoles through novel course. The combination of 1-phenyl-3-ferrocenyl-pyrazole was examined including addition-cyclocondensation like response conditions. The response continued through three phases using of expansion cyclo-buildup of acetyl ferrocene with phenyl hydrazine pursued by cyclizing reagent iodine with $NaHCO_3$. In both syntheses, each time single product isolated having good yields (87 and 79 %). Ferrocenyl substituted pyrazoles were examined by spectroscopic techniques (^{1}H NMR, IR, MS) and their biological properties have been screened.

Keywords: Ferrocenyl substituted pyrazoles, Addition cyclo-condensation, Biological activities.

INTRODUCTION

The science of heterocyclic compounds is the most mind blowing parts of natural science. It is similarly fascinating for its hypothetical ramifications, for the assorted variety of its engineered techniques, and for the physiological and modern criticalness of heterocyclic compounds. Specifically, the heterocyclic compounds have been widely contemplated for their characteristic enthusiasm, as well as on the grounds that numerous normal items, numerous medications and drugs and numerous dyestuffs have a place with this gathering. Heterocyclic compounds are cyclic natural substances which contain in the ring framework somewhere around one or more carbons are replaced with other atoms. Numerous alkaloids, nutrients, antitoxins and numerous manufactured medicines and dyestuffs are heterocyclic, thus likewise are numerous substances (for example, nucleic acids, vitamins, *etc*.) which are most associated with the life processes. Probably any particle which can shape two covalent bonds is fit for framing a heterocyclic compound [1,2]. Heterocyclic compounds hold a unique place among pharmaceutical industries. The excellent limit of heterocyclic centers to serve both as biomimetics and as open pharmacophores has, all things considered, added to their novel motivator as standard key segments of different medications. Starting late, unique products, catalysts and reagents have been made for the association of heterocyclic compounds. An expansive number of them encounter the impacts of various drawbacks as usage of toxic reagents, long reaction times, lesser yields and low selectivity. Along these properties, scientists are continuing searching for some reagents which can be used to coordinate new heterocyclic structures with inconceivable identity, higher reactivity and noticeable viability. Five membered heterocycles have a group of such compounds however pyrazole is one of them for research point of view since it is a piece of numerous medications. Pyrazole ring is the primary substance in the structure of helpful pesticides and composts in agribusiness just as in pharmaceutical organizations. Pyrazole and its derivatives have immense natural properties moreover cytotoxic, against malarial, antitumour, antibacterial, antimicrobial action and calming, antiproliferatie, hostile to angiogenics narcotic sleep inducing activities [3-9]. In decades ago, pyrazole compounds are progressively underlined on account of their various properties. Pyrazole and its related compounds are very dynamic in medication structuring as a result of their distinctive

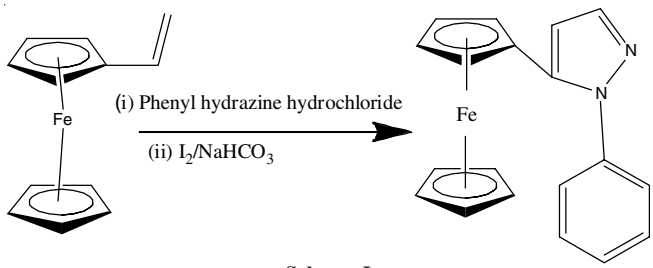
organic exercises like enemy of angiogenic, anti-inflammatory, antibacterial activities, *etc*. Therapeutic scientific experts are likewise open to the incorporation of ferrocene into their medication plan systems due to the uniqueness presented by its essence. Ferrocene is a steady, non-toxic compound and having great redox properties. Present research is proceeding to plan new compounds which are dynamic against a wide scope of malignant growths and have lesser symptoms [10,11]. Ferrocenes are likewise known to display a wide scope of natural movement and furthermore ferrocene has pulled in unique consideration since it is an impartial artificially steady and non-poisonous. Numerous ferrocenyl compounds show intriguing, cytotoxic, antitumor, antimalarial, hostile to organometallic compounds and DNA separating exercises [12-17]. There are numerous models available in literature to the utilization of ferrocene in tranquilize structure techniques. In one examination on some non-steroidal calming operators, the substitution of fragrant ring by ferrocene did not enhance cells to joint or platelet aggregatory exercises in the subsequent compound [18]. Examinations with ferrocene-containing penicillins, cephalosporins and rifamycins demonstrated that the consideration of ferrocene did not present any uncommon favourable position [19,20]. Then again, critical changes in action profiles had been recorded when ferrocene was brought into set up medications like tamoxifen and chloroquine. Ferrocenyl subordinates are among the most potential mixes which can be utilized in malignant growth examining. Malignancy is a class of malady described by uncontrolled cell expansion and the capacity of these cells to attack different tissues. In 2007, 5-alkyl-2-ferrocenyl-6,7-dihydropyrazolo-[1,5-*a*]pyrazin-4[5*H*]-one complex and its subsidiaries having inhibitory impact towards A549 malignancy cell development was discovered [21]. New ferrocenyl subordinates of pyrazole analogs were set up in 2014. One of them was (2-formyl-1-chlorovinyl)-ferrocene arranged by utilizing acetylferrocene. These mixes have anticancer and organic activities [22].

(*R*)-N,N-dimethyl-1-[(*S*)-2-{3-(1-phenyl)-1*H*-pyrazolyl}-ferrocenyl]ethylamine was combined from 1-(*R*)-N,N-dimethyl-ferrocenylethylamine by Burkhardt and Drommi [23]. This exploration was another methodology in the field of chelating pyrazole-containing ligands [23]. In 2008, Hasinoff *et al*. [24] determined ester coupled bisanthrapyrazole subsidiaries of 7-chloro-2-[2[-[(2-hydroxyethyl)methyl amino]ethyl]anthrax-[1,acd]pyrazole-6(2*H*)-one (APa) have more grounded DNA official and cytotoxic action. The bisintercalation capability of the compound and the quality of DNA restricting was acquired by increment in DNA liquefying temperature [24]. Tan *et al*. [25] integrated the subordinates of novel anthrapyrazole from emodin and found the cytotoxic impact of these towards malignant growth cell. These subsidiaries were gotten by joining different cationic alkyl amino side chains onto a pyrazole ring. Emodin has co-planar structure so it has low DNA restricting partiality and low or inconsequential cytotoxicity against different malignant growth cells.

EXPERIMENTAL

^{1}H NMR spectra was recorded on Bruker Avance II 400 NMR Spectrometer at 400MHz using TMS as internal standard. IR spectra was made utilizing Perkin Elmer-Spectrum RX-IFTIR instrument tests were set up as KBr pellets. Mass spectra (*m/z*) was made by utilizing Gas Chromatography Mass Spectrometry through SAIF LAB, Chandigarh, India. Every single synthetic compounds utilized of investigative grade procured from LOBA, MERCK and OTTO. All the solvents were distilled before use.

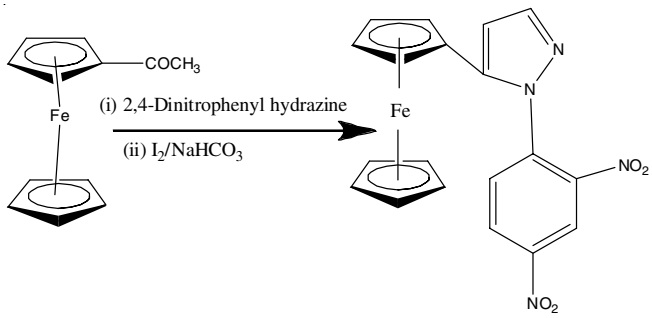
Synthesis of cyclopenta-2,4-dien-1-yl(2-(1-phenyl-1*H*-pyrazol-4-yl)cyclopenta-2,4-dien-1-yl)iron(III): 1-Phenyl-3-ferrocenyl pyrazole was synthesized by using vinyl ferrocene. Vinyl ferrocene was treated with phenyl hydrazine hydrochloride by refluxing at 100 ºC using water bath for 3 h. Desired product was synthesized by treating reaction mixture with iodine crystals (0.001 mol) and (0.02 mol) of sodium bicarbonate by refluxing using magnetic stirrer with hot plate at 60 ºC for 2 h followed by treating hot reaction mixture with ice and a single product was separated out (**Scheme-I**). IR (KBr, ν_{max}, cm^{-1}): 752 (C-H *str*.), 1150 (C-N *str*.), 1666 (C=N *str*.), ^{1}H NMR: (400 MHz, DMSO, in ppm) δ: 6.69-6.79 (m, 5H, Cp), 1.93 (s, 1H, allylic-H), 7.27-7.31 (d, 1H, *cis*-H), 1.99-2.00 (d, 1H, allylic-H), 4.30 (s, 1H, vinyl-H), 2.48 (s, 1H, CH-N), 7.00-7.16 (m, 5H, Ar-H). GC-MS (*m/z*) calcd. (found): 328 (328.17). Anal. calcd. (found) % for $C_{19}H_{17}N_2{}^{56}Fe$: C, 69.51 (69.47); H, 5.18 (5.13); N, 9.14 (9.11).



Scheme-I

Synthesis of cyclopenta-2,4-dien-1-yl(2-(1-(2,4-dinitro)-phenyl-1*H*-pyrazol-4-yl)cyclopenta-2,4-dien-1-yl)iron(III): Nitro derivative of 1-phenyl-3-ferrocenyl pyrazole was synthesized by using acetyl ferrocene. Acetyl ferrocene was treated with 2,4-dinitrophenyl hydrazine by refluxing at 60 ºC using water bath for 3 h. Desired product was synthesized by treating reaction mixture with iodine crystals (0.001 mol) and sodium bicarbonate (0.02 mol) by refluxing using magnetic stirrer with hot plate at 100 ºC for 2.5 h followed by treating hot reaction mixture with ice for quenching the reaction at this stage and a single product was separated out (**Scheme-II**). IR (KBr, ν_{max}, cm^{-1}): 473 (Cp-Fe-Cp *str*.), 742 (C-H *str*.), 1140 (C-N *str*.), 1661(C=N *str*.). ^{1}H NMR: (400MHz, DMSO, in ppm) δ: 6.49-6.57(m, 5H, Cp), 0.83 (s, 1H, allylic-H), 7.66-7.68 (d, 1H, *cis*-H), 0.99-1.00 (d, 1H, allylic-H), 4.30 (s, 1H, vinyl-H), 4.08(s, 1H, CH-N), 7.10-7.16 (m, 5H, Ar-H). GC-MS (*m/z*) calcd. (found): 422 (422.23). Anal. calcd. (found) % for $C_{19}H_{14}N_4O_4{}^{56}Fe$: C, 54.02 (53.99); H, 3.31 (3.31); N, 14.3 (14.21).

Antimicrobial assay: *in vitro* Antimicrobial activity has been evaluated for ferrocenyl substituted pyrazoles against pathogenic strains of bacteria (*Staphylococcus aureus*, *Klebsiella pneumoniae*) and fungi (*Aspergillus niger*, *Trichophyton rubrum*) using disc plate diffusion assay. The stock solutions of both



Scheme-II

synthesized feroocenyl substituted pyrazoles (1000 ppm) were prepared in DMSO. The plates of culture medium using nutrient agar and potato-dextrose agar were prepared for bacterial and fungal growth respectively under sterilized condition. The various concentrations of ferrocenyl substituted pyrazoles (100, 150, 200 and 250 ppm) were loaded on 5 mm sterilized filter paper discs and placed on agar plates followed by incubation at 30 ºC for 24 h and 72 h to evaluate the effect of compound on bacterial and fungal growth, respectively. Neomycin and fluconazole were used as standard antimicrobial agents for bacterial and fungal study, respectively.

DNA photo-cleavage assay: DNA cleavage activity of the synthesized ferrocenyl substituted pyrazoles were studied by agarose gel electrophoresis using supercoiled pUC19 plasmid DNA. The total volume of reaction mixture was 10 µL containing 0.5 µg of plasmid DNA in TE (tris 10 mM, EDTA 0.01 mM, pH 8.0) buffer with various concentrations of synthesized ferrocenyl substituted pyrazoles. The Eppendorfs carrying reaction mixture were placed directly on the surface of a trans-illuminator (8000 mW/cm) at 360 nm for 30 min. After irradiation, samples were further incubated at 37 ºC for 1 h. Irradiated samples were mixed with 6X loading dye containing 0.25 % bromophenol blue and 30 % glycerol. The samples were then analyzed by electrophoresis on a 0.8 % agarose horizontal slab gel in tris-acetate EDTA buffer (40 mM Tris, 20 mM acetic acid, 1 mM EDTA, pH: 8.0) with comparison to untreated plasmid DNA as a control. Gel was stained with ethidium bromide (1 µg/mL) and photographed under UV light.

RESULTS AND DISCUSSION

Antimicrobial activities: The antimicrobial effect of synthesized ferrocenyl substituted pyrazoles towards harmful human pathogenic microorganisms has been studied with comparison to Neomycin and fluconazole. The antimicrobial action of ferrocenyl substituted pyrazoles was measured in term of zone of inhibition (Tables 1 and 2) and MIC values were found to be in the range of 85-95 µg/mL. Previous evidences have supported that pyrazole based derivatives possesses broad spectrum of antimicrobial effects. Bekhit and Abdel-Aziem [26] investigated the antimicrobial effect of novel pyrazole derivatives against Gram-positive and Gram-negative bacterial as well as pathogenic fungi. Mandal *et al*. [27] and Munyaneza *et al*. [28] have also reported the promising antimicrobial activities of metal complexes of pyrazole.

DNA photo-cleavage: The photo-induced DNA cleavage has been carried out using super coiled plasmid DNA by gel electrophoresis [29]. The result of DNA photo-cleavage investigations is shown in Fig. 1. As literature revealed that in plasmid DNA electrophoresis the super coiled DNA (form I) migrate relatively fast with comparison to nicked DNA (form II) [30]. Super coiled form is relaxed to produce a open-circular form which is slower moving if one strand of super coiled form is cleaved. If both strands of super coiled form are cleaved, a

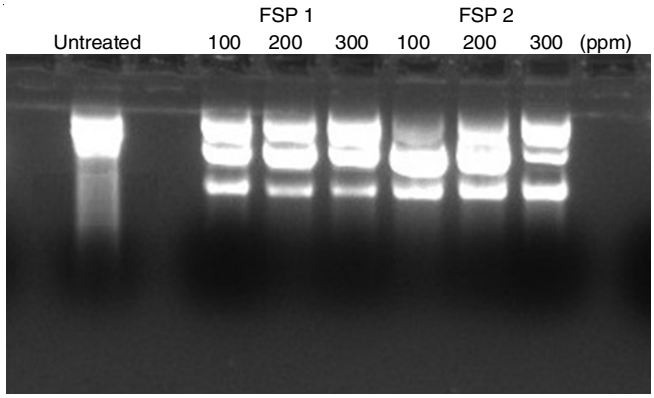


Fig. 1. DNA photo-cleavage activity (FSP = ferrocenyl substituted pyrazoles)

TABLE-1
ANTIMICROBIAL EFFECTS OF SPECTRAL STUDY OF CYCLOPENTA-2,4-DIEN-1-YL(2-(1-PHENYL-1*H*-PYRAZOL-4-YL)CYCLOPENTA-2,4-DIEN-1-YL)IRON(III)

	Bacterial strains								Fungal strains							
	S. aureus				*K. pneumoniaae*				*A. niger*				*Trichophyton rubrum*			
Conc. (ppm)	100	150	200	250	100	150	200	250	100	150	200	250	100	150	200	250
Zone of inhibition (mm)	12± 0.5	16± 0.7	17± 0.6	18± 0.6	11± 0.5	13± 0.5	18± 0.5	20± 0.7	10± 0.5	12± 0.5	15± 0.5	18± 0.5	09± 0.7	12± 0.4	14± 0.5	18± 0.6
Standards	21 mm (Neomycin)				23 mm (Neomycin)				24 mm (Fluconazole)				9 mm (Fluconazole)			

TABLE-2
ANTIMICROBIAL EFFECTS OF CYCLOPENTA-2,4-DIEN-1-YL(2-(1-(2,4-DINITRO)PHENYL-1*H*-PYRAZOL-4-YL)CYCLOPENTA-2,4-DIEN-1-YL)IRON(III)

	Bacterial strains								Fungal strains							
	S. aureus				*K. pneumoniaae*				*A. niger*				*Trichophyton rubrum*			
Conc. (ppm)	100	150	200	250	100	150	200	250	100	150	200	250	100	150	200	250
Zone of inhibition (mm)	12± 0.5	15± 0.7	17± 0.6	21± 0.6	10± 0.5	14± 0.5	16± 0.5	19± 0.7	10± 0.5	13± 0.5	16± 0.5	18± 0.5	09± 0.7	12± 0.4	15± 0.5	17± 0.6
Standards	21 mm (Neomycin)				23 mm (Neomycin)				24 mm (Fluconazole)				9 mm (Fluconazole)			

nicked form is generated which migrate in between the super coiled and open circular form [31,32]. The conversion of form I to form II was observed with the treatment of both synthesized ferrocenyl substituted pyrazoles in comparison to untreated plasmid DNA which indicates that ferrocenyl substituted pyrazoles have significant DNA-cleavage potential. Han *et al*. [33] have reported the role of pyrazole based ligands as promising DNA cleaving action *via* hydrolytic mechanisms. Feng *et al*. [34] have also investigated DNA binding capabilities of pyrazole deri-vatives and suggested their promising role in designing the chemotherapeutic drugs.

Conclusion

In the present study, two ferrocenyl substituted pyrazoles have been designed and synthesized by means of novel course and characterized. The IR, ^{1}H NMR and mass data confirmed the proposed structure and geometry. It is obvious from the confirmations that such holding is possible. The antimicrobial study shown that both ferrocenyl substituted pyrazoles are strong antimicrobial agents and further DNA photocleavage investigation confirmed that both compounds have good DNA cleavage potential.

ACKNOWLEDGEMENTS

The authors are thankful to Sophisticated Analytical Instrumentation Facility (SAIF), Punjab University, Chandigarh, India for providing the necessary instrumental support.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. L.C. Behr, R. Fusco and C.H. Jarboe, ed.: R.H. Wiley, The Chemistry of Heterocyclic Compounds: Pyrazoles, Pyrazolines, Pyrazolidines, Indazoles and Condensed Rings, Interscience Publishers: New York, vol. 3 (1967).
2. G.M. Badger, The Chemistry of Heterocyclic Compounds, Academic Press: New York, vol. 5 (1961).
3. R. Mukherjee, *Coord. Chem. Rev.*, **203**, 151 (2000); https://doi.org/10.1016/S0010-8545(99)00144-7.
4. P. Braunstein and F. Naud, *Angew. Chem. Int. Ed.*, **40**, 680 (2001); https://doi.org/10.1002/1521-3773(20010216)40:4<680::AID-ANIE6800>3.0.CO;2-0.
5. S. Trofimenko, *J. Am. Chem. Soc.*, **88**, 1842 (1966); https://doi.org/10.1021/ja00960a065.
6. S. Trofimenko, *J. Am. Chem. Soc.*, **89**, 3170 (1967); https://doi.org/10.1021/ja00989a017.
7. S. Trofimenko, *J. Am. Chem. Soc.*, **89**, 6288 (1967); https://doi.org/10.1021/ja01000a053.
8. S. Trofimenko, *Chem. Rev.*, **72**, 497 (1972); https://doi.org/10.1021/cr60279a003.
9. K. Karrouchi, S. Radi,Y. Ramli, J. Taoufik,Y.N. Mabkhot, F.A. Al-Aizari and M. Ansar, *Molecules*, **23**, 134 (2018); https://doi.org/10.3390/molecules23010134.
10. S.S. Braga and A.M.S. Silva, *Organometallics*, **32**, 5626 (2013); https://doi.org/10.1021/om400446y.
11. K.J. Nikula, J.D. Sun, E.B. Barr, W.E. Bechtold, P.J. Haley, J.M. Benson, A.F. Eidson, D.G. Burt, A.R. Dahl, R.F. Henderson, I.Y. Chang, J.L. Mauderly, M.P. Dieter and C.H. Hobbs, *Official J. Soc. Toxicol.*, **21**, 127 (1993); https://doi.org/10.1093/toxsci/21.2.127.
12. L.A. Summers, *Adv. Heterocycl. Chem.*, **35**, 281 (1984); https://doi.org/10.1016/S0065-2725(08)60151-8.
13. M. Bioani and M. Gonzalez, *Mini Rev. Med. Chem.*, **5**, 409 (2005); https://doi.org/10.2174/1389557053544047.
14. K.Y. Lee, J.M. Kim and J.N. Kim, *Tetrahedron Lett.*, **44**, 6737 (2003); https://doi.org/10.1016/S0040-4039(03)01648-4.
15. K.S. Jain, T.S. Schitre, P.B. Miniyar, M.K. Kathiravan, V.S. Bendre, V.S. Veer, S.R. Shahane and C.J. Shishoo, *Curr. Sci. (India)*, **90**, 793 (2006).
16. Z. Jin, *Nat. Prod. Rep.*, **23**, 464 (2006); https://doi.org/10.1039/b502166a.
17. T. Pinho e Melo, *Curr. Org. Chem.*, **9**, 925 (2005); https://doi.org/10.2174/1385272054368420.
18. B.E. Maryanoff, S.L. Keeley and F.J. Persico, *J. Med. Chem.*, **26**, 226 (1983); https://doi.org/10.1021/jm00356a020.
19. D. Scutaru, L. Tataru, I. Mazilu, E. Diaconu, T. Lixandru and C. Simionescu, *J. Organomet. Chem.*, **401**, 81 (1991); https://doi.org/10.1016/0022-328X(91)86197-X.
20. D. Scutaru, I. Mazilu, L. Tataru, M. Vata and T. Lixandru, *J. Organomet. Chem.*, **406**, 183 (1991); https://doi.org/10.1016/0022-328X(91)83185-7.
21. Y. Xia, Z.W. Dong, B.X. Zhao, X. Ge, N. Meng, D.S. Shin and J.-Y. Miao, *Bioorg. Med. Chem.*, **15**, 6893 (2007); https://doi.org/10.1016/j.bmc.2007.08.021.
22. S. Kumar, *J. Biosci.*, **2**, 60 (2014).
23. U. Burkhardt, D. Drommi and A. Togni, *Inorg. Chim. Acta*, **296**, 183 (1999); https://doi.org/10.1016/S0020-1693(99)00391-6.
24. B.B. Hasinoff, H. Liang, X. Wu, L.J. Guziec, F.S. Guziec Jr., K. Marshall and J.C. Yalowich, *Bioorg. Med. Chem.*, **16**, 3959 (2008); https://doi.org/10.1016/j.bmc.2008.01.033.
25. J.H. Tan, Q.X. Zhang, Z.S. Huang, Y. Chen, X.D. Wang, L.Q. Gu and J.Y. Wu, *Eur. J. Med. Chem.*, **41**, 1041 (2006); https://doi.org/10.1016/j.ejmech.2006.04.006.
26. A.A. Bekhit and T. Abdel-Aziem, *Bioorg. Med. Chem.*, **12**, 1935 (2004); https://doi.org/10.1016/j.bmc.2004.01.037.
27. S. Mandal, M. Mondal, J.K. Biswas, D.B. Cordes, A.M.Z. Slawin, R.J. Butcher, M. Saha and N. Chandra Saha, *J. Mol. Struct.*, **1152**, 189 (2018); https://doi.org/10.1016/j.molstruc.2017.09.015.
28. A. Munyaneza, G. Kumar and I.C. Morobe, *Synth. Catal.*, **3**, 1 (2018); https://doi.org/10.4172/2574-0431.100020.
29. Y.-J. Liu, H. Chao, L.-F. Tan, Y.-X. Yuan, W. Wei and L.-N. Ji, *J. Inorg. Biochem.*, **99**, 530 (2005); https://doi.org/10.1016/j.jinorgbio.2004.10.030.
30. M. Kumar, H.S. Pallvi, H.S. Tuli and R. Khare, *Asian J. Chem.*, **31**, 799 (2019); https://doi.org/10.14233/ajchem.2019.21732.
31. M. Patel, M. Chhasatia and B. Bhatt, *Med. Chem. Res.*, **20**, 220 (2011); https://doi.org/10.1007/s00044-010-9310-9.
32. Q.L. Zhang, J.G. Liu, J.Z. Liu, H. Li, Y. Yang, H. Xu, H. Chao and L.N. Ji, *Inorg. Chim. Acta*, **339**, 34 (2002); https://doi.org/10.1016/S0020-1693(02)00923-4.
33. C. Han, Y.C. Guo, D.D. Wang, X.J. Dai, F.J. Wu, H.F. Liu, G.F. Dai and J.C. Tao, *Chin. Chem. Lett.*, **26**, 534 (2015); https://doi.org/10.1016/j.cclet.2015.01.006.
34. J. Feng, H. Qi, X. Sun, S. Feng, Z. Liu, Y. Song and X. Qiao, *Chem. Pharm. Bull. (Tokyo)*, **66**, 1065 (2018); https://doi.org/10.1248/cpb.c18-00546.